REMARKS

Amendment to the Claims

The claims have been amended to be limited to the elected species. Applicant expressly reserves the right to pursue the non-elected inventions in divisional applications, without the need to file a terminal disclaimer.

Rejection of Claims 25 and 44-46 Under 35 U.S.C. § 103:

The Examiner has rejected Claims 25 and 44-46 under 35 U.S.C. §103, contending that these claims are unpatentable over Braxton in view of the teachings of Boissel et al., Weich et al. and Lin et al. The Examiner contends that Braxton teaches the making of PEGylated cysteine variants, and teaches that a cysteine can be inserted into the sequence at positions of the protein that are surface exposed and not part of the active site of the protein, and further, that Braxton lists erythropoietin (EPO) as a useful protein for such modification. The Examiner acknowledges that Braxton does not teach or suggest the addition of a C-terminal cysteine to EPO or therefore the attachment of PEG at that site. However, the Examiner contends that the art teaches that the C-terminal four residues of EPO are not required for function, citing Boissel et al. (page 15989), and citing Weich et al. for allegedly teaching that attaching additional protein sequence to the C-terminus of EPO resulted in a protein with bioactivity. Lin et al. is cited for the teaching of the sequence represented by SEQ ID NO:2. Accordingly, the Examiner contends that it would have been obvious that the C-terminus of EPO is not required for activity and to add a cysteine at the C-terminus for the attachment of a PEG.

The Examiner's rejection of Claims 25 and 44-46 under 35 U.S.C. §103 is respectfully traversed. Contrary to the Examiner's position that the art at the time of the invention teaches that the C-terminal residues of EPO are not required for protein activity, it is Applicants' position that the literature at the time of the invention is contradictory as to whether the C-terminus of EPO is important for biological activity of the protein, and in fact, provides several demonstrations that the C-terminus is important for biological activity of the protein. Moreover, as discussed below, cysteine is the only amino acid capable of forming disulfide bonds, rendering it unique in its ability to disrupt protein structure, and also rendering the effects of cysteine insertions in a protein unpredictable with respect to studies of other types of

substitutions or deletions. Accordingly, it is Applicants' position that the combination of references cited by the Examiner fails to teach or suggest the presently claimed invention.

More specifically, with respect to the teachings in the art regarding the biological activity of residues at the C-terminus of EPO, Boissel et al. is cited as an example of a report suggesting that the C-terminus of EPO is non-essential for biological activity, due to teachings that deleting amino acids 162-166 of EPO or substituting a KDEL sequence tag or a polyHis sequence tag for amino acids 162-166 of EPO did not alter EPO biological activity.

However, as shown in the attached reference, Quelle et al. obtained completely different results using different C-terminal amino acid tags. Quelle et al. used a similar approach as Boissell et al. to construct EPO analogs in which amino acids 162-166 of EPO were replaced by amino acids encoding a consensus phosphorylation epitope or a hemagglutinin epitope tag, but reported that *both* EPO analogs were *inactive* in biological assays (see Fig. 5 and page 466, col. 1). Quelle et al. thus concluded that the C-terminus of EPO is essential for biological activity of the protein (page 466, col. 1, last sentence of first paragraph).

Fibi et al. (also enclosed) used an immunological approach to determine that the C-terminus was essential for EPO biological activity. Fibi et al. immunized rabbits with small peptides derived from several regions of the EPO protein, and found that only the peptide comprising amino acids 152-166 of EPO stimulated development of antibodies capable of neutralizing EPO bioactivity *in vitro*, leading these authors to conclude that the C-terminal region of EPO was essential for biological activity of the protein (see Abstract and page 1205, column 2, paragraph beginning on line 5). Therefore, the fact that some modifications at the C-terminus of EPO result in an active protein does not indicate that a cysteine insertion at the C-terminus will yield a protein with full or wild-type activity, and indeed, there are examples in the literature teaching that the C-terminus of EPO is important for biological activity and that modifications at the C-terminus can disrupt protein activity.

Moreover, as discussed above, given that cysteine is unique in its ability to disrupt protein structure, one can not use examples of modification with other amino acids to predict the effect of a cysteine modification at the same site. As one example of several, the attached reference by Olins et al., shows that when libraries of random amino acid substitutions were created in another growth hormone superfamily member, interleukin-3 (IL-3), and the mutant proteins were expressed in *E. coli*, the IL-3 muteins varied significantly in their activities using

an *in vitro* cell proliferation assay (see Table II, page 23757). In almost every case, different amino acid substitutions at the same amino acid position yielded mutant proteins with quite different bioactivities. Applicants can provide other literature examples supporting this position, but it is believed that this reference illustrates the point.

The art also indicates that proper disulfide bond formation is critical for EPO bioactivity. For example, Elliot et al reported that disruption of the C7-C161 disulfide bond in EPO by substituting Ser for C7 or Ser for C161 results in EPO proteins possessing severely altered structures and possessing less than 2% of wild type EPO bioactivity (Fig. 1 and text on page 497, column 2, lines 28-32. The cysteine residue added by the inventor following R166 is in close proximity to C161 and therefore, based on Elliot et al., one of skill in the art might expect that this insertion would interfere with proper formation of the critical C7-C161 disulfide bond.

In addition, the literature indicates that deletion analyses are not necessarily predictive of the effects of amino acid substitutions or insertions. For example, Wen et al. (enclosed) reported that deletion of amino acids 43-47 of EPO yielded an EPO protein with no significant loss of biological activity (See Fig 1, page 22840). However, Elliott et al. (enclosed) reported that changing K45 to D resulted in an EPO protein that had a greater than 100-fold decrease in biological activity (Fig. 1, page 495; Table 1, page 500, and discussion starting with last paragraph on page 469 and extending to page 470; also see page 499, column 1, last paragraph of Results section). Wen et al. also indicate that amino acids 163-166 of EPO could be deleted without any significant loss of biological activity, yet as discussed above, Quelle et al report that substituting a Hemagglutinin epitope tag or a phosphorylation epitope tag for amino acids 162-166 of EPO yields an inactive EPO protein.

Boissel et al. reported that EPO analogs containing a deletion of amino acids 32-36 possess about 50% of wild type EPO specific activity (see fig. 3, p 15988, and discussion on page 15990, column 2, line 8-12), whereas Lin et al. reports that changing C33 to a proline greatly reduces EPO biological activity (cited by Boissell et al on page 15990, last sentence of column 1, extending to top of column 2).

Bittorf et al. (enclosed) reported that an EPO analog containing a deletion of amino acids 13-17 (referred to as EPO analog in Tables 1 on page 134 and Table 2 on page 136) had wild-type EPO biological activity. In contrast, Elliott et al. reported that EPO analogs in which R14 is

changed to a Q or in which Y15 is changed to an I possess less than 2% of EPO biological activity (page 497, column 2, paragraph 2; and fig. 1, page 495).

In view of the unpredictability of the effect on biological activity of various modifications to the C-terminus of EPO, and the further unpredictability of making cysteine modifications in any protein at the time of the invention, Applicants assert that it would not have been obvious at the time of the invention that an EPO protein with a cysteine inserted at the C-terminus, including a PEGylated EPO protein, would be biologically active. However, as shown in the attached Declaration under 37 CFR 1.132, the inventor has made this protein and has shown that it is biologically active. This result was surprising in view of the art at the time of the invention, as discussed above.

With regard to the Examiner's comments regarding insertions at the N-terminus of EPO, Applicants have several references that show that the literature at the time of the invention contradicts the Examiner's assertion that the N-terminus of EPO is not required for biological activity. However, since the claims have been limited to a cysteine variant having an insertion at the C-terminus, these references and arguments are not believed to relevant to the presently claimed invention.

In view of the foregoing remarks, the Examiner is respectfully requested to withdraw the rejection of Claims 25 and 44-46 under 35 U.S.C. §103.

Applicants have attempted to address all of the Examiner's rejections as set forth in the November 28 Office Action and submit that the claims are in a condition for allowance. Any further concerns regarding Applicants' position should be directed to the below-named agent at (303) 863-9700.

Respectfully submitted,

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Date: 28 February 2007